Chapter 6: Acid Fast Staining Laboratory

Background and Introduction

The **acid-fast stain** is adifferential stain and will be able to inform the microbiologist on the composition of the cell wall of the microbe. Please recall that there are three types of cell walls: Gram Positive, Gram Negative, and Acid Fast. If a microbiologist performs a Gram stain on an acid-fast microbe, the results will be inconclusive.

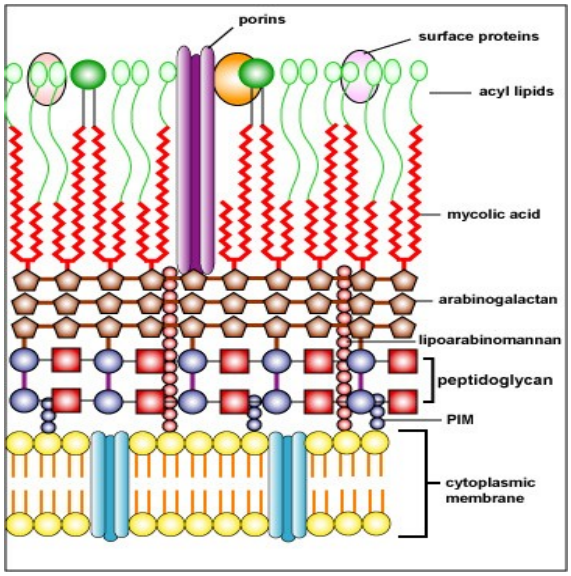
Pre-lab question:

1. Why are the results inconclusive? Explain what kind of stains are used and why they are ineffective.

2. What is the difference between polar and non-polar molecules?

3. Why do Acid-fast microbes need a plasma membrane? Isn't a cell wall good enough?

Since these bacteria are acid-fast and are resistant to the Gram stain, it is important that a microbiologist uses the acid-fast staintechnique to differentiate the cell wall of these types of microorganisms. In the figure below you will see the composition of an Acid-fast microbe. These bacteria have a waxysubstance called **mycolic acid** in theircell walls, which is a non-polar, waxy substance that is impermeable to any polar solution. Theyalso contain polymers calledarabinogalacta,lipoarabinomannan, and peptidoglycans. The microbes that are considered acid-fast include *Mycobacterium* and *Nocardia.* This includes pathogenic organisms such as *M. leprae* and *M. tuberculosis*, which causes Leprosy (Hansen's disease) and Tuberculosis respectively.



The procedure that will be observed in this laboratory experiment is called the Ziehl-Neelsen staining technique. The **primary stain** in the acid-fast staining procedure is **carbol fuchsin**. This will contain **phenol** that should help solubilize the cell wall and ultimately allow the stain to enter the cell. This part of the procedure will require **heat** in order for the stain to penetrate the waxy cell wall.

Pre-lab question:

4. How does heat help the carbol fuschin enter the cell?

Once the carbol fuschin is added with heat, all of the cells will appear as a reddish color. The differential step is going to be the application of the **decolorizer** and in this procedure, the decolorizer solvent is composed of Ethanol and Hydrocholoric Acid. This **decolorizer** will decolorize all cells except for acid-fast cells. The acid alcohol solvent cannot penetrate the non-polar waxy cell wall of acid-fast microbes. The final step is the application of the **counterstain**, which is **methylene blue**. This will stain the decolorized non acid-fast cells blue.

Pre-lab question:

5. Fill out the chart below. Describe the color that is present after the application of each solvent.

Solvent	Acid Fast Color Present	Non Acid Fast Color Present

Materials:

- 1 Wax Pencils

- 1 Distilled Water Bottles

- 1 Disinfectant Bottles

- 1 Test Tube Racks

- 1 Metal Inoculating Loops

- 1 Metal Inoculating Needles 1

- 1 Bunsen Burner and Hose

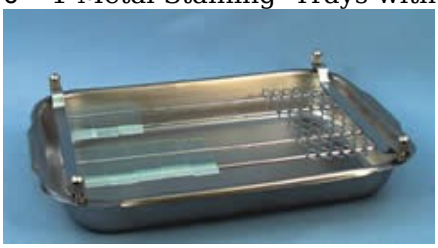
- 1 Boxes of Microscope Slides

- 1 “Waste” 500ml Beakers (1 per table)

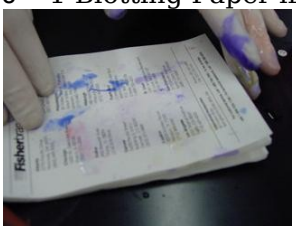
- Acid Fast Staining Kits
  - Each kit should have the following
    - Carbol Fuschin
    - Acid-Alcohol Decolorizer
    - Methylene Blue

- 1 Optical Lens Paper 1

- 1 Metal Staining Trays with Racks



- 1 Blotting Paper if available or 1 Large Brown Paper Towels Roll



- 1 Tupperware

Microorganisms:

Mycobacterium smegmatis
Staphylococcus aureus

Procedure:

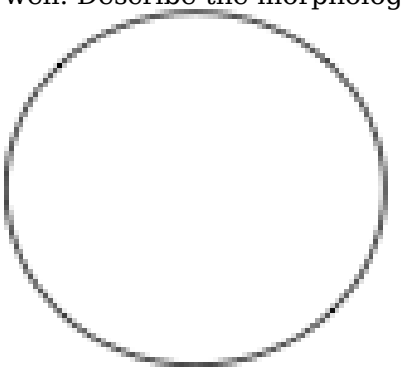
1. Prepare two smears for each microorganism. CAUTION!!! *Mycobacterium smegmatis* does not stick very well on the glass slides. It is important that you add as much of the microorganism onto the slide. After the smear has air dried completely, heat fix the microbes gently.
2. As the smear is drying, obtain a plastic Tupperware.
    a. Add two layers of paper towels in the Tupperware and moisten them.
3. Place the heat fixed smears atop of the moist paper towels. Cover the smears with two strips of paper towels, then flood the surface of these strips with carbol fuchsin.
4. Place the lid on the Tupperware and leave on edge open for venting. Place the entire container in the microwave and heat for 1 minute at 100% power.
5. Remove the container from the microwave and then at your bench top remove the slides from the Tupperware and place it on the staining rack.

CAUTION!!! HOT GLASS LOOKS LIKE COLD GLASS

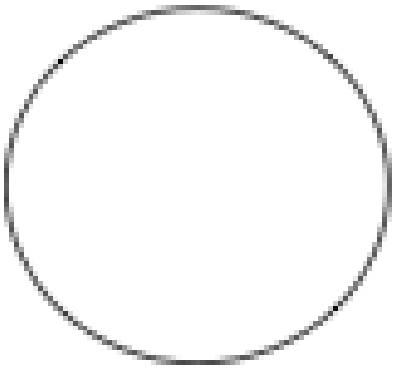
6. Apply the Acid/Alcohol decolorizer by tilting the slide at a 45^0 angle. Allow the acid/alcohol to run across the smear for 15 seconds.
7. Immediately after decolorizing, rinse with dH_2O
8. Apply the methylene blue counter stain by flooding the surface of the smear and allowing this to sit for 1 minute.
9. Rinse the excess methylene blue away with dH_2O and blot the slide dry with bibulous paper or brown paper towels.
10. Observe the microbes using a light microscope at 1000X with oil and record your results below.
11. Clean your microscope with lens cleaner, paying extra attention to the 40X and 100X objectives. Have your instructor check your microscope to make sure it is clean.
12. _____ (Instructor's initials)
13. Dispose of the liquid stain waste in the designated waste receptacle in the front and back of the laboratory. Dispose of glass slides in glass waste container. Disinfect your bench and place all materials back to where you found them. In addition, please give your cultures back to your instructor.

Data/Results

Using colored pencils, draw and label *Staphylococcus species* and *Mycobacterium smegmatis* in the circles below. In addition, write down what you physically see as well. Describe the morphological characteristics of each microbe.



Staphylococcus species at 1000X



Mycobacterium smegmatis species at 1000x

Post-Lab Questions:

1. Why must we use heat when adding the primary stain to the smear during the acid-fast staining protocol?

2. Why do we use acid-alcohol instead of alcohol-acetone mixture as the decolorizing agent during the acid-fast staining protocol?

3. Why is heat not used during the counter stain step of the acid-fast staining protocol?